The consequences of aneuploidy and chromosome instability

Schukken, Klaske Marijke

DOI:
10.33612/diss.135392967

Chapter 7

Summary, Discussions and Future Directions

Summary

Chromosome mis-segregation was first observed over 100 years ago in sea urchin embryos by Dr. Boveri. This mis-segregation was shown to be detrimental to cell and organism physiology, and Dr. Boveri was the first to suggest this chromosome mis-segregation might lead to cancer [210,211]. Today chromosome instability (CIN) is considered a hallmark of cancer [4,9,39,40,183], and large bodies of evidence have shown that the majority of solid tumors are aneuploid[7,8]. Despite years of research, there are still many unknowns and paradoxical effects of CIN and aneuploidy. Lower rates of CIN accelerate tumor progression in a cancer-prone background[4,18,21,38,50], while high rates of CIN are lethal to cancer cells[4,16,31,32,159], and yet other drivers of CIN can provoke cancer formation even in an un-predisposed background[14]. Some tissues tolerate CIN, while others do not[15,38]. Aneuploidy is present in a majority of cancers[7,8], and often lowers the expected survival of patients[11–13,105,107,108], despite the fact that most karyotypes inhibiting cell proliferation[2,3,42]. Investigating which types and levels of CIN help inhibit tumorigenesis in specific tissues, and how cancer cells can deal with the stresses of CIN and aneuploidy may lead to significant advances in CIN cancer therapy.

To better understand some of these apparent paradoxes within the field, CIN and aneuploidy should be assessed separately. In **chapter 2** we review these differences and discuss the various effects of CIN and aneuploidy. How can CIN be both toxic to cells and beneficial to tumor progression? Why are most aneuploidies detrimental to cell growth, yet so common in aggressive cancers? We discuss that the effects CIN has on cells and tissues are largely dependent upon intra and inter-cellular conditions such as tissue type, genetic mutations, level and type of CIN, the specific aneuploid karyotypes that cells develop, length of time cells are experiencing CIN, and whether the cells are in cell culture or *in vivo* conditions. While aneuploidy is frequently measured in both cell lines and in cells isolated from *in vivo* tumors and tissues, CIN is rarely measured *in vivo*. Ultimately, we conclude that more *in vivo* mouse models are needed to quantify CIN rates and cell fate, so that it becomes possible to better understand the effects of CIN, mutations, and the impact of drugs on CIN and aneuploidy *in vivo*.

Looking further into the differences between CIN and aneuploidy, we screened both stable aneuploid cells[2,52], and cells with ongoing chromosome mis-segregations to find drugs that selectively killed either cell

line (**Chapter 3**). We found that the drug(s) that were toxic to CIN cells were not toxic to stable, aneuploid cells, and vice versa. Our results, in combination with previous research[138] indicated that stable aneuploid cells may be especially sensitive to metabolic inhibitors. Our CIN screen, on the other hand, showed that the Src inhibitor SKI606 was synergistically toxic to Spindle Assembly (SAC) knockdown cells. While Src had been previously shown to be essential for microtubule nucleation[151], we expand upon this by showing that Src inhibition has a significant effect on microtubule polymerization rates. Additionally, we find that nocodazole, another drug that alters tubulin stability[212], is similarly toxic to SAC inhibited cells. Thus, we conclude that combining SAC knockdown with a drug that alter spindle dynamics significantly increase chromosome mis-segregation and lead to decreased cell growth.

Much like the drug screen described in **chapter 3**, most CIN and aneuploidy-related research has mostly been performed using cultured cells. While the importance of these experiments should not be understated, cell culture may not directly mimic *in vivo* conditions[1,24,25]. Rates of chromosome mis-segregation, for example, have been shown to increase once cells are cultured *in vitro* [24]. While CIN cancer cell lines have been studied in cell culture, very little research has been done on the rates of chromosome mis-segregation within tumors *in vivo* [1]. One of the main reasons CIN is rarely studied *in vivo* is due to the lack of models that allow such experiments.

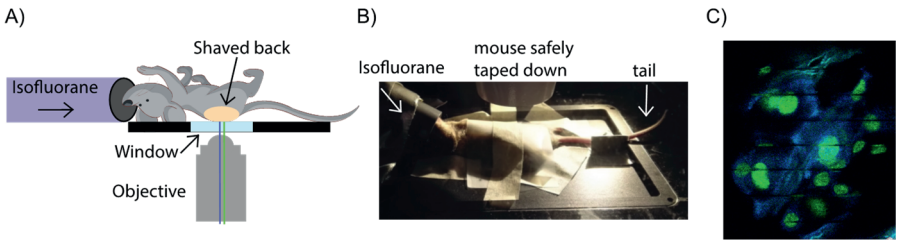

Figure 1: Imaging fluorescent mice. A-C) A diagram (A) and photo (B) of how fluorescent mice are viewed on the two-photon microscope. Mice are kept aneasthesized with isofluorane gas. C) Fluorescent cells within live mouse skin; H2B is displayed in green (Nucleus), and tubulin in blue (cytoplasm).

We engineered a novel mouse model in which one can observe mitosis in living mice (**Chapter 4**). This mouse model, called the "CIN tracker", fluorescently labels both the chromatin, via H2B-eGFP, and the spindle network, via mTurquoise2[173]-αTubulin. Additionally, the mitosis marker cassette can be activated in a tissue-specific manner at a time-point of

choice, allowing for more targeted research. We validated the model by inducing fluorescence within these mice and imaging the skin of living mice, which displayed mosaic fluorescence expression of both the tubulin network and nuclear chromatin (Figure 1). In future experiments, this fluorescent mouse model can be used to monitor *in vivo* CIN rates, both in healthy tissue and tumors. Since the mice are alive during imaging, we do not only observe mitosis, but also the fate of a cell after mis-segregating a chromosome. We would also be able to image a specific tissue multiple times over the course of days, week, months or even years, allowing for the study of long-term effects and changes in the rate and type of chromosome segregation.

The "CIN tracker" mouse model would allow us to visualize mis-segregating chromosomes *in vivo*, but not the aneuploid chromosomes themselves. While the aneuploidy of human tissues and tumors has been studied previously [91,92,183], *in vivo* aneuploidy measurements are end-point measurements[44]. Thus, the fate of aneuploid cells *in vivo* has not been studied. In **chapter 5**, we set out to create an "AneuTracker" mouse model in which we can visualize aneuploidy within living cells and tissues. While we tested a fluorescent dCas9 method to fluorescently label chromosomes, we were unable to find small guide RNAs that localized to specific chromosomes within mouse cells, and we found that extended periods of time expressing dCas9 and sgRNAs was toxic to cells. We had more success using a fluorescent tetR and TRE binding site system. Using this system, we are able to fluorescently label a single chromosome within living cells in cell culture. We then engineered a new mouse model in which the TRE binding tandem was integrated within the genomic DNA. The TRE integration within the mouse genome was analyzed in cell culture and found to be sufficient to produce clear fluorescent chromosomal foci once a fluorescent tetR protein was transduced in the cell. In the future, a new mouse model will need to be made that expresses a fluorescent tet protein, in addition to a chromatin marker to discriminate individual nuclei, and perhaps labeled Tubulin as well. These mice with fluorescent tet proteins can be crossed with the AneuTracker mice to yield a mouse model in which chromosomes are fluorescently labeled with the tetR-TRE binding system. Additionally, the TRE binding site within the mouse was flanked with PiggyBack transposon sites, which would allow the TRE sequence to mobilize throughout the genome if the cells are exposed to the PiggyBac transposase protein. This would allow for the creation of several tagged mouse lines, each with the TRE integrated in another chromosome.

Finally, in **chapter 6**, we look into the effects of inducing a specific form of CIN *in vivo*. Inducing CIN by knocking out the SAC has been shown to be embryonically lethal[40], and toxic to hair follicle stem cells[15], but tolerated in epithelial cells[15], T-cells, and hepatocytes[38]. Here we show that inducing CIN by inactivation of Mad2 or by mutating Mps1 in mammary tissue has no effect on mouse lifespan. By combining these CIN models with a mammary specific P53 knockout model, we show that the in the Mad2 knockout, but not in the Mps1 mutant, tumorigenesis is accelerated in a cancer-prone background. Thus, the driver of CIN is important in determining whether CIN accelerates tumorigenesis.

Since knocking out Mad2 and inducing CIN is embryonic lethal[40], but tolerated in several adult tissues such as the mammary tissue, we investigated whether inducing CIN systemically in adult mice would be tolerated (**chapter 6**). We found that inactivation of Mad2, with and without an accompanying P53 knockout, was rapidly lethal to mice. Within four days mice had lost 15% of their weight. Their intestinal villi were severely affected, with a significant increase in the number of apoptotic cells and a significant decrease in villus length. In addition to a strong phenotype in intestinal villi, we also found that hematopoietic cells showed a phenotypic change, albeit much more modest, while liver, lung and kidneys had no noticeable phenotypic alterations. This shows that the effect of CIN can be highly differential per tissue type, which might depend on the proliferation rate within the tissue.

Discussion and future directions

Future therapies to target CIN

Since CIN is a hallmark of cancer[39,192], and aneuploidy is found within 3 out of 4 tumors[7,8], specifically targeting CIN cells may be an effective therapy against these cancers. In our drug screen (**chapter 3**) we use SAC inhibition, via Mad2 knockdown, to model chromosomal instability. We find one drug that stands out from the rest when it comes to selectively inhibiting CIN cells: SKI606. The Src family inhibitor SKI606 was found to significantly alter microtubule dynamics, and it acts synergistically with SAC inhibition to increase mis-segregation rates dramatically. Nocodazole, a microtubule depolymerase[212] has a similar synergistic toxicity with Mad2 knockdown cells, significantly increasing their CIN rate. While lower CIN can be

tolerated by many cell types, high rates of CIN are known to be toxic to tumor progression [16,31,113]. We show that combining altered microtubule dynamics with the alleviation of the SAC is synergistically toxic, and may be an effective way to target CIN cells.

Src inhibition is known to prevent microtubule nucleation[151], which in turn reduces the number of new microtubules formed. In **chapter 3** we show Src inhibition also increases microtubule plus end polymeration rates, a phenomenon that also occurs when treating with the microtubule depolymerase nocodazole[137]. Decreasing the total number of microtubules in the cell would increase the pool of free-floating tubulin substrate, which in turn may increase microtubule polymerization rates. I hypothesize that cells treated with Src inhibitors or nocodazole have reduced numbers of microtubules and thus form a less dense spindle networks. A less dense spindle network would have an increased chance of faulty microtubule-kinetochore interactions. While the SAC would prevent cells from entering anaphase until the spindle manages to attach to all kinetochores, cells with a (partially) defective SAC and a low-density spindle network would have a greatly increased chance of mis-segregating chromosomes.

Cancer cells rarely display complete loss of SAC fucntionality[39,213], which might mean that Src inhibition would only be effective to a few specific forms of cancer. However, many CIN cancer cells do have altered microtubule dynamics [47,136,137,214,215]. Because of this, inhibiting the SAC within cancers that already have altered microtubule dynamics may target those cancer cells. As personalized medicine becomes more prevalent within the clinic, screening patients for cancers who have altered microtubule dynamics or a complete loss of SAC may be a successful approach to determine which patients would benefit from SAC inhibiting and/or Src inhibiting medications during chemotherapy.

Combining SAC inhibition with altered microtubule dynamics in order to induce high rates of CIN [32,164] is already being examined in stage 3 clinical trials as a method to target CIN cancers [165–167]. In these clinical trials, SAC inhibition (via Mps1 inhibitors) is combined with the microtubule stabilizing drug paclitaxel [66,137]. While paclitaxel decreases microtubule polymerization rates, Src inhibition increases this (chapter 3). While those drugs therefore have opposite effects on microtubule dynamics, they both alter spindle function, both significantly increase CIN in SAC inhibited cells, and are both toxic to SAC inhibited cells, suggesting that SAC inhibition and deregulation

microtubule polymerization in general is synergistically toxic to cells. While this thesis therefore supports the clinical trials looking into the synergistic paclitaxel and Mps1 inhibitor toxicity, it also reveals there are multiple drug combinations that have this synergistic toxicity. While more research is needed before our findings can be used in the clinic, targeting Src inhibited cells with SAC inhibitors, or combining SAC inhibition with nocodazole may prove to be equally effective as paclitaxel. Alternatively, using other microtubule destabilizing compounds such as the commonly used chemotherapeutic Vincristine, or Src inhibitors in combination with Mad2 inhibition may become a secondary line of treatment for cancers which have become resistant to paclitaxel and Mps1 inhibitors.

Mouse models to visualize CIN and aneuploidy in vivo

Since CIN and aneuploidy are different concepts with different consequences (**chapter 2**), and different targetable pathways (**chapter 3**), it is important that we have models that would allow us to monitor CIN, as well as models that would allow us to monitor aneuploidy. We therefore engineered, the "CIN tracker" allowing to monitor chromosome mis-segregation *in vivo* by fluorescently labeling the chromatin and the spindle network (**chapter 4**), and a separate "AneuTracker" model was made in order to visualize aneuploidy of a specific chromosome within a living tissue (**chapter 5**).

Chromosome mis-segregation has rarely been measured *in vivo*, largely due to the lack of mouse models designed for such purposes. Unfortunately, the level of CIN, and the fate of CIN cells in cell culture may not reflect what is actually happening within an organism [1,24]. The CIN tracker model would allow us to view the level of ongoing chromosome-mis-segregation and the fate of CIN cells within different tumors, and the effect of drug treatments on CIN rates.

In addition, the "AneuTracker", can be used to visualize the frequency of the copy number alterations for a specific chromosome in various tissues over time, and the rate of chromosome-specific aneuploidy within certain tumors. While aneuploidy has frequently been measured *in vivo*, these results are always endpoint measurements. This new model will allow us to measure the aneuploidy of a living tissue over time, observe the effects of treatments and mutations and as a consequence, lower the number of mice that are necessary per experiment.

While designed for a specific purpose, both models have broader applications as well. These models can be used to view the nuclear structure or tubulin dynamics within living tissues, they could be used for lineage tracing, and the derivation of fluorescent organoids or primary cell lines. These mouse models are an important next step for bringing many *in vitro* results and studies into an *in vivo* context, so that the consequences of CIN and aneuploidy within both healthy tissues and cancers can be better understood.

Final conclusions

CIN and aneuploidy are complex phenomena that have a large impact on cell physiology caused by a large number of deregulated processes. The mouse models for inducible CIN described in chapter 6 show how tissue types can determine a cell's response to CIN. The drug screen described in Chapter 3 does not only help highlight the different vulnerabilities imposed by CIN and aneuploidy, but also reveals how the rate of CIN determines how a cell population is affected, as we find that increasing CIN beyond a tolerable threshold may be a very effective way to target CIN cells.

Finally, this thesis presents two new mouse models: the "CIN tracker" mouse, to monitor chromosome mis-segregation in living tissues and cancers, and the "AneuTracker", to monitor chromosome copy numbers for an individual chromosome in living tissue. The "CIN tracker" model has already been shared with several collaborators, while the "AneuTracker" muse model still needs the reporter cassette mouse to be generated, which we expect to finish in the next few years. Together, these beyond state of the art mouse models will significantly aid further work on the dynamics and consequences of CIN and aneuploidy within a living organism.

Bibliography

Bibliography

1. Schukken KM, Foijer F. CIN and Aneuploidy: Different Concepts, Different Consequences. *BioEssays*. 2018;40(1). doi:10.1002/bies.201700147
2. Sheltzer JM, Ko JH, Replogle JM, Passerini V, Storchova Z, Amon A. Single-chromosome Gains Commonly Function as Tumor Suppressors. *Cancer Cell*. 2017;31(2):240-255. doi:10.1016/j.ccell.2016.12.004
3. Ben-david U, Arad G, Weissbein U, et al. Aneuploidy induces profound changes in gene expression, proliferation and tumorigenicity of human pluripotent stem cells. *Nat Commun*. 2014;5:1-11. doi:10.1038/ncomms5825
4. Simon JE, Bakker B, Foijer F. CINcere Modelling: What Have Mouse Models for Chromosome Instability Taught Us? *Recent results cancer res*. 2015;200:39-60. doi:10.1007/978-3-319-20291-4_2
5. Biancotti JC, Narwani K, Mandefro B, et al. The in vitro survival of human monosomies and trisomies as embryonic stem cells. *Stem Cell Res*. 2012;9(3):218-224. doi:10.1016/j.scr.2012.07.002
6. Rutledge SD, Douglas TA, Nicholson JM, et al. Selective advantage of trisomic human cells cultured in non-standard conditions. *Sci Rep*. 2016;6(February):22828. doi:10.1038/srep22828
7. Duijf PHG, Schultz N, Benezra R. Cancer cell predominantly loose small chromosomes. *Int J Cancer*. 2013;7132(10):2316-2326. doi:10.1007/s11103-011-9767-z.Plastid
8. Weaver BA, Cleveland DW. Does aneuploidy cause cancer? *Curr Opin Cell Biol*. 2006;18(6):658-667. doi:10.1016/j.ceb.2006.10.002
9. Giam M, Rancati G. Aneuploidy and chromosomal instability in cancer: a jackpot to chaos. *Cell Div*. 2015;10(1):3. doi:10.1186/s13008-015-0009-7
10. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-674. doi:10.1016/j.cell.2011.02.013
11. Carter SL, Eklund AC, Kohane IS, Harris LN, Szallasi Z. A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers. *Nat Genet*. 2006;38(9):1043-1048. doi:10.1038/ng1861
12. Lennartz M, Minner S, Brasch S, et al. The combination of DNA

ploidy status and PTEN/6q15 deletions provides strong and independent prognostic information in prostate cancer. *Clin Cancer Res*. 2016;22(11):2802-2811. doi:10.1158/1078-0432.CCR-15-0635

13. Otsu H, Iimori M, Ando K, et al. Gastric Cancer Patients with High PLK1 Expression and DNA Aneuploidy Correlate with Poor Prognosis. *Oncology*. 2016;91(1):31-40. doi:10.1159/000445952
14. Levine MS, Bakker B, Boeckx B, et al. Centrosome amplification is sufficient to promote spontaneous tumorigenesis in mammals. *Dev Cell*. 2017;in press(3):313-322.e5. doi:10.1016/j.devcel.2016.12.022
15. Foijer F, DiTommaso T, Donati G, et al. Spindle checkpoint deficiency is tolerated by murine epidermal cells but not hair follicle stem cells. *PNAS*. 2013;110(8):2928-2933. doi:10.1073/pnas.1217388110
16. Silk AD, Zasadil LM, Holland AJ, Vitre B, Cleveland DW. Chromosome missegregation rate predicts whether aneuploidy will promote or suppress tumors. *PNAS*. 2013;110(44):4134-4141. doi:10.1073/pnas.1317042110
17. Nicholson JM, Cimini D. Cancer Karyotypes: Survival of the Fittest. *Front Oncol*. 2013;3(June):148. doi:10.3389/fonc.2013.00148
18. Foijer F, Albacker LA, Yue Y, et al. Deletion of the Mad2 spindle checkpoint gene causes chromosome instability with differential effects on liver cancer and lymphoma. *Cancer Cell*. 2007;11(1):9-23. doi:10.1016/j.ccr.2006.10.019
19. Dai W, Wang Q, Liu T, et al. Slippage of Mitotic Arrest and Enhanced Tumor Development in Mice with BubR1 Haploinsufficiency Advances in Brief Slippage of Mitotic Arrest and Enhanced Tumor Development in Mice with. *Cancer Res*. 2004;64:440-445. doi:10.1158/0008-5472.CAN-03-3119
20. Dekanty A, Barrio L, Muzzopappa M, Auer H, Milán M. Aneuploidy-induced delaminating cells drive tumorigenesis in Drosophila epithelia. *PNAS*. 2012;109(50):20549-20554. doi:10.1073/pnas.1206675109
21. Sotillo R, Hernando E, Díaz-rodríguez E, Teruya-feldstein J, Lowe SW, Benezra R. Mad2 overexpression promotes aneuploidy and tumorigenesis in mice. *Cancer Cell*. 2007;11(1):9-23.

22. Bakhoum SF, Ngo B, Laughney AM, et al. Chromosomal instability drives metastasis through a cytosolic DNA response. *Nature*. 2018;553(7689):467-472. doi:10.1038/nature25432
23. de Oliveira Mann CC, Kranzusch PJ. cGAS Conducts Micronuclei DNA Surveillance. *Trends Cell Biol*. 2017. doi:10.1016/j.tcb.2017.08.007
24. Roemeling-van Rhijn M, de Klein A, Douben H, et al. Culture Expansion Induces Non-Tumorigenic Aneuploidy In Adipose Tissue-Derived Mesenchymal Stromal Cells. *Cytotherapy*. 2013;15(11):1352-1361. doi:10.1016/j.jcyt.2013.07.004
25. Dekel-Naftali M, Aviram-Goldring A, Litmanovitch T, et al. Screening of human pluripotent stem cells using CGH and FISH reveals low-grade mosaic aneuploidy and a recurrent amplification of chromosome 1q. *Eur J Hum Genet*. 2012;20(12):1248-1255. doi:10.1038/ejhg.2012.128
26. Sansregret L, Swanton C. The Role of Aneuploidy in Cancer Evolution. *CSH Perspect*. 2017;7:1-18. doi:10.1101/cshperspect.a028373
27. Bakker B, Taudt A, Belderbos M, et al. Single cell sequencing reveals karyotype heterogeneity in murine and human tumours. *Genome Biol*. 2016;17:1-15. doi:10.1186/s13059-016-0971-7
28. Lee AJX, Endesfelder D, Rowan AJ, et al. Chromosomal Instability Confers Intrinsic Multi-Drug Resistance. *Cancer Res*. 2011;71(5):1858-1870. doi:10.1158/0008-5472.CAN-10-3604.Chromosomal
29. Sotillo R, Schvartzman J, Socci ND, Benezra R, Biology C, Program G. Mad2-induced chromosome instability leads to lung tumor relapse after oncogene withdrawal. *Nature*. 2010;464(7287):436-440. doi:10.1038/nature08803.Mad2-induced
30. Tijhuis AE, Johnson SC, Mcclelland SE. The emerging links between chromosomal instability (CIN), metastasis , inflammation and tumour immunity. *Mol Cytogenes*. 2019;12(17):1-21.
31. Zasadil LM, Britigan EMC, Ryan SD, et al. High rates of chromosome missegregation suppress tumor progression, but do not inhibit tumor initiation. *Mol Biol Cell*. 2016;27(608):1981-1989. doi:10.1091/mbc.E15-10-0747
32. Maia ARR, Linder S, Song J, et al. Mps1 inhibitors synergise with low doses of taxanes in promoting tumour cell death by enhancement of errors in cell division. *Br J Cancer*. 2018;(March).

doi:10.1038/s41416-018-0081-2
INTRODUCTION10.1038/s41416-018-0081-2
33. Golas JM, Arndt K, Etienne C, et al. SKI-606 , a 4-Anilino-3-quinolinecarbonitrile Dual Inhibitor of Src and Abl Kinases , Is a Potent Antiproliferative Agent against Chronic Myelogenous Leukemia Cells in Culture and Causes Regression of K562 Xenografts in Nude Mice. *Cancer Res*. 2003;63:375-381.
34. Boschelli DH, Ye F, Wang YD, et al. Optimization of 4-Phenylamino-3-quinolinecarbonitriles as Potent Inhibitors of Src Kinase Activity. *J Med Chem*. 2001;44:3965-3977. doi:10.1021/jm0102250
35. Roukos V, Burgess RC, Misteli T. Generation of cell-based systems to visualize chromosome damage and translocations in living cells. *Nat Protoc*. 2014;9(10):2476-2492. doi:10.1038/nprot.2014.167
36. Ma H, Naseri A, Reyes-gutierrez P, Wolfe SA, Zhang S, Pederson T. Multicolor CRISPR labeling of chromosomal loci in human cells. *PNAS*. 2015;112(10):3002-3007. doi:10.1073/pnas.1420024112
37. Ma H, Tu L, Naseri A, Huisman M, Zhang S, Grunwald D. Multiplexed labeling of genomic loci with dCas9 and engineered sgRNAs using CRISPRainbow. *Nat Biotechnol*. 2016;34(5):528-531. doi:10.1038/nbt.3526
38. Foijer F, Albacker LA, Bakker B, et al. Deletion of the MAD2L1 spindle assembly checkpoint gene is tolerated in mouse models of acute T-cell lymphoma and hepatocellular carcinoma. *Elife*. 2017;6:1-22. doi:10.7554/eLife.20873
39. Foijer F. CINister thoughts. *Biochem Soc Trans*. 2010;38(6):1715-1721. doi:10.1042/BST0381715
40. Foijer F, Draviam VM, Sorger PK. Studying chromosome instability in the mouse. *Biochim Biophys Acta*. 2008;1786(1):73-82. doi:10.1016/j.bbcan.2008.07.004
41. Valind A, Jin Y, Baldetorp B, Gisselsson D. Whole chromosome gain does not in itself confer cancer-like chromosomal instability. *PNAS*. 2013;110(52):21119-21123. doi:10.1073/pnas.1311163110
42. Williams BR, Prabhu VR, Hunter KE, et al. Aneuploidy affects proliferation and spontaneous immortalization in mammalian cells. *Science*. 2008;322(5902):703-709. doi:10.1126/science.1160058

43. Stingele S, Stoehr G, Peplowska K, Cox J, Mann M, Storchova Z. Global analysis of genome, transcriptome and proteome reveals the response to aneuploidy in human cells. *Mol Syst Biol.* 2012;8(1):608. doi:10.1038/msb.2012.40
44. Bakker B, Bos H Van Den, Lansdorp PM, Foijer F. How to count chromosomes in a cell: an overview of current and novel technologies. *BioEssays.* 2015;37(5):570-577. doi:10.1002/bies.201400218
45. Musacchio A, Salmon ED. The spindle-assembly checkpoint in space and time. *Nat Rev Mol Cell Biol.* 2007;8(5):379-393. doi:10.1038/nrm2163
46. Bortoletto E, Mognato M, Ferraro P, et al. Chromosome instability induced in the cell progeny of human T lymphocytes irradiated in G(0) with gamma-rays. *Mutagenesis.* 2001;16(6):529-537. http://www.ncbi.nlm.nih.gov/pubmed/11682645.
47. Thompson SL, Bakhoum SF, Compton DA. Mechanisms of Chromosomal Instability. *Curr Opin Cell Biol.* 2010;20(6):1-23. doi:10.1016/j.cub.2010.01.034.Mechanisms
48. Thompson SL, Compton DA. Examining the link between chromosomal instability and aneuploidy in human cells. *J Cell Biol.* 2008;180(4):665-672. doi:10.1083/jcb.200712029
49. Kanda T, Sullivan KF, Wahl GM. Histone-GFP fusion protein enables sensitive analysis of chromosome dynamics in living mammalian cells. *Curr Biol.* 1998;8(7):377-385. doi:10.1016/S0960-9822(98)70156-3
50. Foijer F, Xie SZ, Simon JE, et al. Chromosome instability induced by Mps1 and p53 mutation generates aggressive lymphomas exhibiting aneuploidy-induced stress. *PNAS.* 2014;111(37):13427-13432. doi:10.1073/pnas.1400892111
51. Nicholson JM, Macedo JC, Mattingly AJ, et al. Chromosome mis-segregation and cytokinesis failure in trisomic human cells. *Elife.* 2015;4. doi:10.7554/eLife.05068
52. Passerini V, Ozeri-Galai E, de Pagter MS, et al. The presence of extra chromosomes leads to genomic instability. *Nat Commun.* 2016;7:10754. doi:10.1038/ncomms10754
53. Clevers H. Modeling Development and Disease with Organoids. *Cell.* 2016;165(7):1586-1597. doi:10.1016/j.cell.2016.05.082
54. Drost J, van Jaarsveld RH, Ponsioen B, et al. Sequential cancer mutations in cultured human intestinal stem cells. *Nature.*

2015;521(7550):43-47. doi:10.1038/nature14415
55. Bakhoum SF, Silkworth WT, Nardi IK, Nicholson JM, Compton DA, Cimini D. The mitotic origin of chromosomal instability. *Biophys Chem*. 2014;24(4):R148-R149. doi:10.1016/j.immuni.2010.12.017.Two-stage
56. Kops GJPL, Foltz DR, Cleveland DW. Lethality to human cancer cells through massive chromosome loss by inhibition of the mitotic checkpoint. *PNAS*. 2004;101(23):8699-8704. doi:10.1073/pnas.0401142101
57. Burds AA, Lutum AS, Sorger PK. Generating chromosome instability through the simultaneous deletion of Mad2 and p53. *PNAS*. 2005;102(32):11296-11301. doi:10.1073/pnas.0505053102
58. Liu Y, Nielsen CF, Yao Q, Hickson ID. The origins and processing of ultra fine anaphase DNA bridges. *Curr Opin Genet Dev*. 2014;26:1-5. doi:10.1016/j.gde.2014.03.003
59. Gelot C, Magdalou I, Lopez BS. Replication stress in mammalian cells and its consequences for mitosis. *Genes (Basel)*. 2015;6(2):267-298. doi:10.3390/genes6020267
60. Tillement V, Remy M-H, Raynaud-Messina B, Mazzolini L, Haren L, Merdes A. Spindle assembly defects leading to the formation of a monopolar mitotic apparatus. *Biol Cell*. 2009;101(1):1-11. doi:10.1042/BC20070162
61. Battini L, Macip S, Fedorova E, et al. Loss of polycystin-1 causes centrosome amplification and genomic instability. *Hum Mol Genet*. 2008;17(18):2819-2833. doi:10.1093/hmg/ddn180
62. Maiato H, Logarinho E. Mitotic spindle multipolarity without centrosome amplification. *Nat Cell Biol*. 2014;16(5):386-394. doi:10.1038/ncb2958
63. Kalatova B, Jesenska R, Hlinka D, Dudas M. Tripolar mitosis in human cells and embryos: occurrence, pathophysiology and medical implications. *Acta Histochem*. 2015;117(1):111-125. doi:10.1016/j.acthis.2014.11.009
64. Normand G, King RW. Understanding cytokinesis failure. *Adv Exp Med Biol*. 2010;675:27-55. doi:10.1007/978-1-4419-6199-0_3
65. Fujiwara T, Bandi M, Nitta M, Ivanova E V, Bronson RT, Pellman D. Cytokinesis failure generating tetraploids promotes tumorigenesis in p53-null cells. *Nature*. 2005;437(7061):1043-1047. doi:10.1038/nature04217
66. Bekier ME, Fischbach R, Lee J, Taylor WR. Length of mitotic

arrest induced by microtubule-stabilizing drugs determines cell death after mitotic exit. *Mol Cancer Ther*. 2009;8(6):1646-1654. doi:10.1158/1535-7163.MCT-08-1084
67. Fenech M, Kirsch-Volders M, Natarajan AT, et al. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. *Mutagenesis*. 2011;26(1):125-132. doi:10.1093/mutage/geq052
68. Huang Y, Fenech M, Shi Q. Micronucleus formation detected by live-cell imaging. *Mutagenesis*. 2011;26(1):133-138. doi:10.1093/mutage/geq062
69. Abe T, Fujimori T. Reporter Mouse Lines for Fluorescence Imaging. *Dev Growth Differ*. 2013;55(4):390-405. doi:10.1111/dgd.12062
70. Janssen A, Beerling E, Medema R, van Rheenen J. Intravital FRET Imaging of Tumor Cell Viability and Mitosis during Chemotherapy. *PLoS One*. 2013;8(5). doi:10.1371/journal.pone.0064029
71. Orth JD, Kohler RH, Foijer F, Sorger PK, Weissleder R, Mitchison TJ. Analysis of mitosis and antimitotic drug responses in tumors by In Vivo microscopy and single-cell pharmacodynamics. *Cancer Res*. 2011;71(13):4608-4616. doi:10.1158/0008-5472.CAN-11-0412
72. Friedberg EC, Henning K, Lambert C, et al. Microcell-mediated chromosome transfer: a strategy for studying the genetics and molecular pathology of human hereditary diseases with abnormal responses to DNA damage. *Basic Live Sci*. 1990;52:257-267.
73. Upender MB, Habermann JK, McShane LM, et al. Chromosome Transfer Induced Aneuploidy Results in Complex Dysregulation of the Cellular Transcriptome in Immortalized and Cancer Cells. *Cancer Res*. 2004;64(19):6941-6969. doi:10.1002/dev.21214.Developmental
74. Oromendia AB, Dodgson SE, Amon A. Aneuploidy causes proteotoxic stress in yeast. *Genes Dev*. 2012;26(24):2696-2708. doi:10.1101/gad.207407.112.Compton
75. Santaguida S, Vasile E, White E, Amon A. Aneuploidy-induced cellular stresses limit autophagic degradation. *Genes Dev*. 2015;29(19):2010-2021. doi:10.1101/gad.269118.115.
76. Torres EM, Williams BR, Tang Y, Amon A. Thoughts on Aneuploidy. *Cold Spring Harb Symp Quant Biol*. 2010;75(617):445-

451. https://www.ncbi.nlm.nih.gov/pubmed/21289044.
77. Pavelka N, Rancati G, Zhu J, et al. Aneuploidy confers quantitative proteome changes and phenotypic variation in budding yeast. *Nature*. 2010;468(7321):321-325. doi:10.1038/nature09529.Aneuploidy
78. Torres EM, Williams BR, Amon A. Aneuploidy: Cells losing their balance. *Genetics*. 2008;179(2):737-746. doi:10.1534/genetics.108.090878
79. Estrada JC, Torres Y, Benguria A, et al. Human mesenchymal stem cell-replicative senescence and oxidative stress are closely linked to aneuploidy. *Cell Death Dis*. 2013;4(691). doi:10.1038/cddis.2013.211
80. Didion JP, Buus RJ, Naghashfar Z, Threadgill DW, Morse HC, de Villena FP-M. SNP array profiling of mouse cell lines identifies their strains of origin and reveals cross-contamination and widespread aneuploidy. *BMC Genomics*. 2014;15(1):847. doi:10.1186/1471-2164-15-847 1471-2164-15-847 [pii]
81. Roschke A V., Tonon G, Gehlhaus KS, et al. Karyotypic Complexity of the NCI-60 Drug-Screening Panel. *Cancer Res*. 2003;63(24):8634-8647.
82. Rondón-Lagos M, Verdun Di Cantogno L, Marchiò C, et al. Differences and homologies of chromosomal alterations within and between breast cancer cell lines: a clustering analysis. *Mol Cytogenet*. 2014;7(1):8. doi:10.1186/1755-8166-7-8
83. Adey A, Burton JN, Kitzman JO, et al. The haplotype-resolved genome and epigenome of the aneuploid HeLa cancer cell line. *Nature*. 2013;500(7461):207-211. doi:10.1038/nature12064
84. Yang S, Lin G, Tan Y, et al. Tumor progression of culture-adapted human embryonic stem cells during long-term culture. *Genes Chromosom Cancer*. 2008;47(8):665-679.
85. Baker DEC, Harrison NJ, Maltby E, et al. Adaptation to culture of human embryonic stem cells and oncogenesis in vivo. *Nat Biotechnol*. 2007;25(2):207-215. doi:10.1038/nbt1285
86. Pfau SJ, Silberman RE, Knouse KA, Amon A. Aneuploidy impairs hematopoietic stem cell fitness and is selected against in regenerating tissues in vivo. *Genes Dev*. 2016;30(12):1395-1408. doi:10.1101/gad.278820.116
87. Pack SD, Weil RJ, Vortmeyer AO, et al. Individual adult human neurons display aneuploidy: Detection by fluorescence in situ

hybridization and single neuron PCR. *Cell Cycle*. 2005;4(12):1758-1760. doi:10.4161/cc.4.12.2153
88. Rehen SK, Yung YC, McCreight MP, et al. Constitutional Aneuploidy in the Normal Human Brain. *J Neurosci*. 2005;25(9):2176-2180. doi:10.1523/JNEUROSCI.4560-04.2005
89. Duncan AW, Newell AEH, Smith L, et al. Frequent Aneuploidy Among Normal Human Hepatocytes. *Gastroenterology*. 2012;142(1):25-28. doi:10.1097/OPX.0b013e3182540562.The
90. Duncan AW, Taylor MH, Hickey RD, et al. The ploidy conveyor of mature hepatocytes as a source of genetic variation. *Nature*. 2010;467(7316):707-710. doi:10.1038/nature09414
91. van den Bos H, Spierings DCJ, Taudt A, et al. Single-cell whole genome sequencing reveals no evidence for common aneuploidy in normal and Alzheimer's disease neurons. *Genome Biol*. 2016;17(1):1-9. doi:10.1186/s13059-016-0976-2
92. Knouse KA, Wu J, Whittaker CA, Amon A. Single cell sequencing reveals low levels of aneuploidy across mammalian tissues. *PNAS*. 2014;111(37):13409-13414. doi:10.1073/pnas.1415287111
93. Cai X, Evrony GD, Lehmann HS, et al. Single-Cell, Genome-wide Sequencing Identifies Clonal Somatic Copy-Number Variation in the Human Brain. *Cell Rep*. 2014;8(5):1280-1289. doi:10.3816/CLM.2009.n.003.Novel
94. Wu J, Springett A, Morris JK. Survival of trisomy 18 (Edwards syndrome) and trisomy 13 (Patau Syndrome) in England and Wales: 2004–2011. *Am J Med Genet*. 2013;161(10):2512-2518. doi:10.1002/ajmg.a.36127
95. Roizen NJ, Patterson D. Down's syndrome. *Lancet*. 2003;361(9365):1281-1289. doi:10.1016/s0140-6736(03)12987-x
96. Gropp A, Winking H, Herbst EW, Claussen CP. Murine trisomy: Developmental profiles of the embryo, and isolation of trisomic cellular systems. *J Exp Zool*. 1983;228(2):253-269. doi:10.1002/jez.1402280210
97. Santaguida S, Richardson A, Iyer DR, et al. Chromosome Mis-segregation Generates Cell- Cycle-Arrested Cells with Complex Karyotypes that Are Eliminated by the Immune System. *Dev Cell*. 2017;41(6):638-651.e5. doi:10.1016/j.devcel.2017.05.022
98. Andriani GA, Almeida VP, Faggioli F, et al. Whole Chromosome Instability induces senescence and promotes SASP. *Sci Rep*.

2016;6(35218).
99. Dobles M, Liberal V, Scott ML, Benezra R, Sorger PK. Chromosome missegregation and apoptosis in mice lacking the mitotic checkpoint protein Mad2. *Cell*. 2000;101(6):635-645. doi:10.1016/S0092-8674(00)80875-2
100. Poulton JS, Cuningham JC, Peifer M. Centrosome and spindle assembly checkpoint loss leads to neural apoptosis and reduced brain size. *J Cell Biol*. 2017;216(5):1255-1265. doi:10.1083/jcb.201607022
101. Gogendeau D, Siudeja K, Gambarotto D, Pennetier C, Bardin AJ, Basto R. Aneuploidy causes premature differentiation of neural and intestinal stem cells. *Nat Commun*. 2015;6:8894. doi:10.1038/ncomms9894
102. Mackenzie KJ, Carroll P, Martin C-A, et al. cGAS surveillance of micronuclei links genome instability to innate immunity. *Nat Publ Gr*. 2017. doi:10.1038/nature23449
103. López-otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The Hallmarks of Aging. *Cell*. 2013;153(6):1194-1217. doi:10.1016/j.cell.2013.05.039.The
104. Carter SL, Eklund AC, Kohane IS, Harris LN, Szallasi Z. A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers. *Nat Genet*. 2006;38(9):1043-1048. doi:10.1038/ng1861
105. Xu J, Huang L, Li J. DNA aneuploidy and breast cancer : a meta-analysis of 141 , 163 cases Study selection. *Oncotarget*. 2016;72(37).
106. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *N Engl J Med*. 2016;374(23):2209-2221. doi:10.1056/NEJMoa1516192
107. Meyer R, Freitag-Wolf S, Blindow S, Büning J, Habermann JK. Combining aneuploidy and dysplasia for colitis' cancer risk assessment outperforms current surveillance efficiency: a meta-analysis. *Int J Colorectal Dis*. 2016;32:1-12. doi:10.1007/s00384-016-2684-5
108. Rubio Bueno P, Naval Gias L, García Delgado R, Domingo Cebollada J, Díaz González F. Tumor DNA content as a prognostic indicator in squamous cell carcinoma of the oral cavity and tongue base. *Head Neck*. 1998;20(3):232-239.
109. Chretien M-L, Corre J, Lauwers-Cances V, et al. Understanding the role of hyperdiploidy in myeloma prognosis: which trisomies

really matter? *Blood*. 2005;17(126):2713-2719.
110. Dziadziuszko R, Wegrzynowicz E, Kardas I, Limon J, Jassem J. Prognostic significance of aneuploidy in non-small cell lung cancer (NSCLC) patients. *J Clin Oncol*. 2004;22(14):7319-7319. doi:10.1200/jco.2004.22.14_suppl.7319
111. Kim J, Kim Y-T, Kim S-H, Lee C-H, Chung DJ, Cho NH. Prognostic value of nuclear DNA quantification and cyclin A in epithelial ovarian cancer. *Gynecol Cancer*. 2001;22(14):5106-5106. doi:10.1200/jco.2004.22.14_suppl.5106
112. Michel LS, Liberal V, Chatterjee A, Sorger PK, Murty VVVS, Benezra R. Mad2 haplo-insufficiency causes premature anaphase and chromosome instability in mammalian cells. *Nature*. 2001;409(6818):355-359.
113. Funk LC, Zasadil LM, Weaver BA. Living in CIN: Mitotic Infidelity and Its Consequences for Tumor Promotion and Suppression. *Dev Cell*. 2016;39(6):638-652. doi:10.1016/j.devcel.2016.10.023
114. Rowald K, Mantovan M, Passos J, et al. Negative Selection and Chromosome Instability Induced by Mad2 Overexpression Delay Breast Cancer but Facilitate Oncogene-Independent Outgrowth. *Cell Rep*. 2016;15(12):2679-2691. doi:10.1016/j.celrep.2016.05.048
115. Bloomfield M, Duesberg P. Inherent variability of cancer-specific aneuploidy generates metastases. *Mol Cytogenet*. 2016;9:90. doi:10.1186/s13039-016-0297-x
116. McGranahan N, Burrell RA, Endesfelder D, Novelli MR, Swanton C. Cancer chromosomal instability: therapeutic and diagnostic challenges. *EMBO Rep*. 2012;13(6):528-538. doi:10.1038/embor.2012.61
117. Lushnikova T, Bouska A, Odvody J, Dupont WD, Eischen CM. Aging mice have increased chromosome instability that is exacerbated by elevated Mdm2 expression. *Oncogene*. 2011;30(46):4622-4631. doi:10.1038/nbt.3121.ChIP-nexus
118. Forsberg LA, Rasi C, Razzaghian HR, et al. Age-related somatic structural changes in the nuclear genome of human blood cells. *Am J Hum Genet*. 2012;90(2):217-228. doi:10.1016/j.ajhg.2011.12.009
119. Baker DJ, Jeganathan KB, Cameron JD, et al. BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. *Nat Genet*. 2004;36(7):744-749. doi:10.1038/ng1382

120. Baker DJ, Dawlaty MM, Wijshake T, et al. Increased expression of BubR1 protects against aneuploidy and cancer and extends healthy lifespan. *Nat Cell Biol*. 2013;15(1):96-102. doi:10.1038/ncb2643
121. Hanks S, Coleman K, Reid S, et al. Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in BUB1B. *Nat Genet*. 2004;36(11):1159-1161. doi:10.1038/ng1449
122. Hanks S, Coleman K, Summersgill B, et al. Comparative genomic hybridization and BUB1B mutation analyses in childhood cancers associated with mosaic variegated aneuploidy syndrome. *Cancer Lett*. 2006;239(2):234-238. doi:10.1016/j.canlet.2005.08.006
123. Matsuura S, Matsumoto Y, Morishima K, et al. Monoallelic BUB1B mutations and defective mitotic-spindle checkpoint in seven families with premature chromatid separation (PCS) syndrome. *Am J Med Genet*. 2006;104(4).
124. Baker DJ, Perez-Terzic C, Jin F, et al. Opposing roles for p16Ink4a and p19Arf in senescence and ageing caused by BubR1 insufficiency. *Nat Cell Biol*. 2008;10(7):825-836. doi:10.1038/ncb1744
125. Baker DJ, Wijshake T, Tchkonia T, et al. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature*. 2011;479(7372):232-236. doi:10.1038/nature10600
126. Baar MP, Brandt RMC, Putavet DA, et al. Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging. *Cell*. 2017;169(1):132-147.e16. doi:10.1016/j.cell.2017.02.031
127. Baker DJ, Jeganathan KB, Malureanu L, Perez-Terzic C, Terzic A, Van Deursen JMA. Early aging-associated phenotypes in Bub3/Rae1 haploinsufficient mice. *J Cell Biol*. 2006;172(4):529-540. doi:10.1083/jcb.200507081
128. Chen M, Huang J-D, Deng HK, et al. Overexpression of eIF-5A2 in mice causes accelerated organismal aging by increasing chromosome instability. *BMC Cancer*. 2011;11(1):199. doi:10.1186/1471-2407-11-199
129. Davoli T, Xu AW, Mengwasser KE, et al. Cumulative haploinsufficiency and triplosensitivity drive aneuploidy patterns and shape the cancer genome. *Cell*. 2013;155(4):948-962. doi:10.1016/j.cell.2013.10.011
130. McGranahan N, Burrell RA, Endesfelder D, Novelli MR,

Swanton C. Cancer chromosomal instability: therapeutic and diagnostic challenges. *EMBO Rep.* 2012;13(6):528-538. doi:10.1038/embor.2012.61
131. Walther A, Houlston R, Tomlinson I. Association between chromosomal instability and prognosis in colorectal cancer : a meta-analysis. *Gut.* 2008;57:941-950. doi:10.1136/gut.2007.135004
132. Torres EM, Williams BR, Tang Y-C, Amon A. Thoughts on aneuploidy. *Cold Spring Harb Symp Quant Biol.* 2010;75:445-451. doi:10.1101/sqb.2010.75.025
133. Zhang C, Spektor A, Cornils H, et al. Chromothripsis from DNA damage in micronuclei. *Nature.* 2015;522:179-184. doi:10.1038/nature14493
134. Gordon DJ, Resio B, Pellman D. Causes and consequences of aneuploidy in cancer. *Nat Rev Genet.* 2012;13(3):189-203. doi:10.1038/nrg3123
135. Pfau SJ, Amon A. Chromosomal instability and aneuploidy in cancer: from yeast to man. *EMBO Rep.* 2012;13(6):515-527. doi:10.1038/embor.2012.65
136. Stolz A, Ertych N, Bastians H. A phenotypic screen identifies microtubule plus end assembly regulators that can function in mitotic spindle orientation. *Cell Cycle.* 2015;14(6):827-837.
137. Ertych N, Stolz A, Stenzinger A, et al. Increased Microtubule assembly rates mediate chromosomal instability in colorectal cancer cells. *Nat Cell Biol.* 2014;16(8):779-791. doi:10.1038/ncb2994
138. Tang Y, Williams BR, Siegel JJ, Amon A. The energy and proteotoxic stress-inducing compounds AICAR and 17-AAG antagonize proliferation in aneuploid cells. *Cell.* 2011;144(4):499-512.
139. Bakhoum SF, Kabeche L, Compton DA, Powell SN, Bastians H. Mitotic DNA damage response: at the crossroads of structural and numberical cancer chromosome instabilities. *Trends Cancer.* 2018;3(3):225-234. doi:10.1016/j.trecan.2017.02.001.Mitotic
140. Bakhoum SF, Kabeche L, Murnane JP, Zaki BI, Compton DA. DNA-Damage Response during Mitosis Induces Whole-Chromosome Missegregation. *Cancer Discov.* 2014;(November):1281-1290. doi:10.1158/2159-8290.CD-14-0403
141. Tang Y-C, Williams BR, Siegel JJ, Amon A. Identification of

aneuploidy-selective antiproliferation compounds. *Cell*. 2011;144(4):499-512. doi:10.1016/j.cell.2011.01.017
142. Soto M, Raaijmakers J, Bakker B, et al. p53 Prohibits Propagation of Chromosome Segregation Errors that Produce Structural Aneuploidies. *Cell Rep*. 2017;19(12):2423-2431. doi:10.1016/j.celrep.2017.05.055
143. Zhao W, Sachsenmeier K, Zhang L, Sult E, Hollingsworth R e., Yang H. A new bliss independence model to analyze drug combination data. *J Biomol Screen*. 2014;(February). doi:10.1177/1087057114521867
144. Worrall JT, Tamura N, Mazzagatti A, et al. Non-random Mis-segregation of Human Chromosomes. *Cell Rep*. 2018. doi:10.1016/j.celrep.2018.05.047
145. Thomas SM, Brugge JS. Cellular Function Regulated by Src Family Kinases. *Annu Rev Cell Dev Bio*. 1997;13:513-609.
146. Dittmann K, Mayer C, Kehlbach R, Rodemann HP. Radiation-induced caveolin-1 associated EGFR internalization is linked with nuclear EGFR transport and activation of DNA-PK. *Mol Cancer*. 2008;9:16-18. doi:10.1186/1476-4598-7-69
147. Destaing O, Sanjay A, Itzstein C, et al. The Tyrosine Kinase Activity of c-Src Regulates Actin Dynamics and Organization of Podosomes in Osteoclasts. *Mol Biol Cell*. 2008;19(January):394-404. doi:10.1091/mbc.E07
148. Nakayama Y, Matsui Y, Takeda Y, et al. c-Src but not Fyn promotes proper spindle orientation in early prometaphase. *J Biol Chem*. 2012;287(30):24905-24915. doi:10.1074/jbc.M112.341578
149. Janssen A, Burg M Van Der, Szuhai K, Kops GJPL. Chromosome Segregation Errors as a Cause of DNA Damage and Structural. *Science*. 2011;333(September):1895-1899.
150. Meraldi P, Draviam VM, Sorger PK. Timing and checkpoints in the regulation of mitotic progression. *Dev Cell*. 2004;7:45-60. doi:10.1016/j.devcel.2004.06.006
151. Colello D, Reverte CG, Ward R, et al. Androgen and Src signaling regulate centrosome activity. *J Cell Sci*. 2010;123(12):2094-2102. doi:10.1242/jcs.057505
152. Stepanova T, Slemmer J, Hoogenraad CC, et al. Visualization of Microtubule Growth in Cultured Neurons via the Use of EB3-GFP (End-Binding Protein 3-Green Fluorescent Protein). *J Neurosci*. 2003;23(7):2655-2664.

153. Ohashi A, Ohori M, Iwai K, et al. Aneuploidy generates proteotoxic stress and DNA damage concurrently with p53-mediated post-mitotic apoptosis in SAC-impaired cells. *Nat Commun*. 2015;6:1-16. doi:10.1038/ncomms8668
154. Bakker B, Terra J, Zhou L, et al. Single cell DNA sequencing reveals distinct molecular types of basal cell carcinoma with unique transcriptome features. *BioRxiv*. 2018. doi:https://doi.org/10.1101/492199
155. Kukharskyy V, Sulimenko V, Macurek L, Sulimenko T, Draberova E, Draber P. Complexes of gamma -tubulin with nonreceptor protein tyrosine kinases Src and Fyn in differentiating P19 embryonal carcinoma cells. *Exp Cell Res*. 2004;298(298):218-228. doi:10.1016/j.yexcr.2004.04.016
156. Nakayama Y, Soeda S, Ikeuchi M, Kakae K, Yamaguchi N. Cytokinesis Failure Leading to Chromosome Instability in v-Src-Induced Oncogenesis. *Int J Mol Sci*. 2017;18(4). doi:10.3390/ijms18040811
157. Smolinski MP, Bu Y, Clements J, et al. Discovery of Novel Dual Mechanism of Action Src Signaling and Tubulin Polymerization Inhibitors (KX2-391 and KX2-361). *J Med Chem*. 2018;61:4704-4719. doi:10.1021/acs.jmedchem.8b00164
158. Liu T, Hu W, Dalton HJ, et al. Targeting Src and Tubulin in Mucinous Ovarian Carcinoma. *Clin Cancer Res*. 2013;01(17):6532-6544. doi:10.1158/1078-0432.CCR-13-1305
159. Janssen A, Kops GJPL, Medema RH. Elevating the frequency of chromosome mis-segregation as a strategy to kill tumor cells. *PNAS*. 2009;106(45).
160. Tannous BA, Kerami M, Stoop PM Van Der, et al. Effects of the Selective MPS1 Inhibitor MPS1-IN-3 on Glioblastoma Sensitivity to Antimitotic Drugs. *JNCI*. 2013;105(17). doi:10.1093/jnci/djt168
161. Bargiela-Iparraguirre; B, Prado-Marchal; L, Pajuelo-Lozano; N, Jimenez; B, Perona; R, Sanchez-Perez I. Mad2 and BubR1 modulates tumourigenesis and paclitaxel response in MKN45 gastric cancer cells. *Cell Cycle*. 2014;13(22):3590-3601.
162. Jemaá M, Galluzzi L, Kepp O, et al. Characterization of novel MPS1 inhibitors with preclinical anticancer activity. *Cell Death Differ*. 2013;20:1532-1545. doi:10.1038/cdd.2013.105
163. Wengner AM, Siemeister G, Koppitz M, et al. Novel Mps1

Kinase Inhibitors with Potent Antitumor Activity. *Mol Cancer Ther*. 2016;(18):583-593. doi:10.1158/1535-7163.MCT-15-0500
164. Maia ARR, Man J De, Boon U, et al. Inhibition of the spindle assembly checkpoint kinase TTK enhances the efficacy of docetaxel in a triple-negative breast cancer model. *Ann Oncol*. 2015;26(July):2180-2192. doi:10.1093/annonc/mdv293
165. Bayer. Phase I Study of Oral BAY 1217389 in Combination With Intravenous Paclitaxel. 2015. https://clinicaltrials.gov/ct2/show/NCT02366949?term=mps1&cond=cancer&rank=3.
166. Institut de Recherches Internationales Servier, ADIR. S 81694 Plus Paclitaxel in Metastatic Breast Cancer. 2018. https://clinicaltrials.gov/ct2/show/NCT03411161?term=mps1&cond=cancer&rank=2.
167. Boston-Pharmaceuticals. Study of Paclitaxel in Combination With BOS172722 in Patients With Advanced Nonhaematologic Malignancies. 2017. https://clinicaltrials.gov/ct2/show/NCT03328494?term=mps1&cond=cancer&rank=1.
168. Haynes W. Bonferroni Correction. In: *Encyclopedia of Systems Biology*. Springer Science; 2013.
169. Chapman PJ, James DI, Watson AJ, Hopkins G V, Waddell ID, Ogilvie DJ. IncucyteDRC : An R package for the dose response analysis of live cell imaging data [version 1 ; referees : 2 approved]. *F1000Research*. 2016;962(5):1-9. doi:10.12688/f1000research.8694.1
170. Jaccard N, Griffin LD, Keser A, et al. Automated Method for the Rapid and Precise Estimation of Adherent Cell Culture Characteristics from Phase Contrast Microscopy Images. *Biotechnol Bioeng*. 2014;111(3):504-517. doi:10.1002/bit.25115
171. Tinevez J, Perry N, Schindelin J, et al. TrackMate : An open and extensible platform for single-particle tracking. *Methods*. 2017;115:80-90. doi:10.1016/j.ymeth.2016.09.016
172. Wickham H. ggplot2: Elegant Graphics for Data Analysis. 2016. http://ggplot2.org.
173. Goedhart J, von Stetten D, Noirclerc-Savoye M, et al. Structure-guided evolution of cyan fluorescent proteins towards a quantum yield of 93%. *Nat Commun*. 2012;3:751. doi:10.1038/ncomms1738
174. Knouse KA, Lopez KE, Bachofner M, et al. Chromosome

Segregation Fidelity in Epithelia Article Chromosome Segregation Fidelity in Epithelia Requires Tissue Architecture. *Cell*. 2018;175(1):200-211.e13. doi:10.1016/j.cell.2018.07.042
175. Hadjantonakis A, Papaioannou VE. Dynamic in vivo imaging and cell tracking using a histone fluorescent protein fusion in mice. *BMC Biotechnol*. 2004;14:1-14. doi:10.1186/1472-6750-4-33
176. Zhang Y V., White BS, Shalloway DI, Tumbar T. Stem cell dynamics in mouse hair follicles: A story from cell division counting and single cell lineage tracing. *Cell Cycle*. 2010;9(8):1504-1510. doi:10.4161/cc.9.8.11252
177. Roth S, Franken P, Monkhorst K, Kong J, Fodde R. Generation and characterization of an inducible transgenic model for studying mouse esophageal biology. *BMC Dev Biol*. 2012;12(1):1. doi:10.1186/1471-213X-12-18
178. Radcliffe P a, Mitrophanous K a. Multiple gene products from a single vector: 'self-cleaving' 2A peptides. *Gene Ther*. 2004;11(23):1673-1674. doi:10.1038/sj.gt.3302361
179. Liu Z, Chen O, Wall JBJ, Zheng M, Zhou Y. Systematic comparison of 2A peptides for cloning multi-genes in a polycistronic vector. *Sci Rep*. 2017;9(2193):1-9. doi:10.1038/s41598-017-02460-2
180. WN M, S G, L W. Dermal architecture and collagen type distribution. *Arch Dermatol Res*. 1977;21(259):1-10.
181. Cox G, Kable E. Second-Harmonic Imaging of Collagen. *Methods Mol Biol*. 2006;319:15-35.
182. Ricke RM, van Deursen JM. Aneuploidy in health, disease, and aging. *J Cell Biol*. 2013;201(1):11-21. doi:10.1083/jcb.201301061
183. Bolhaqueiro ACF, Ponsioen B, Bakker B, et al. Ongoing Chromosomal Instability and karyotype evolution in human colorectal cancer organoids. *Nat Genet*. 2019;51(May):824-834. doi:10.1038/s41588-019-0399-6
184. Domingues PH, Nanduri LSY, Seget K, et al. Cellular prion protein PrPC and ecto-5'-nucleotidase are markers of the cellular stress response to aneuploidy. *Cancer Res*. 2017;77(11):2914-2926. doi:10.1158/0008-5472.CAN-16-3052
185. Candéias SM, Gaipl US. The Immune System in Cancer Prevention , Development and Therapy. *Anticancer Agents Med Chem*. 2016;16(1):101-107.
186. Cavallo F, De Giovanni C, Nanni P, Forni G, Lollini P-L. 2011:

the immune hallmarks of cancer. *Cancer Immunol Immunother*. 2011;60(3):319-326. doi:10.1007/s00262-010-0968-0
187. Vasudevan A, Baruah PS, Smith JC, et al. Single chromosome gains can function as metastasis suppressors and metastasis promoters. *BioRxiv*. 2019.
188. Gao C, Su Y, Koeman J, et al. Chromosome instability drives phenotypic switching to metastasis. *PNAS*. 2016;113(51):14793-14798. doi:10.1073/pnas.1618215113
189. van Ree JH, Zhou W, van Deursen JM. Transgenesis in Mouse Embryonic Stem Cells. *Methods Mol Biol*. 2011;693:143-162.
190. Sanger. *Chimera Mouse Production by Blastocyst Injection*. https://www.eummcr.org/protocols/mouse-production_chimera-mouse-production-by-blastocyst-injection.pdf.
191. Mitelman F, Johansson B, Mertens F. Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer. Cancer Genome Anatomy Project.
192. Naylor RM, van Deursen JM. Aneuploidy in Cancer and Aging. *Annu Rev Genet*. 2016;50(1):45-66. doi:10.1146/annurev-genet-120215-035303
193. Soutoglou E, Dorn JF, Sengupta K, et al. Positional stability of single double-strand breaks in mammalian cells. *Nat Cell Biol*. 2007;9(6):675-682. doi:10.1038/ncb1591
194. Aguilera A, García-Muse T. Causes of genome instability. *Annu Rev Genet*. 2013;47(July):1-32. doi:10.1146/annurev-genet-111212-133232
195. Gelfand Y, Rodriguez A, Benson G. TRDB — The Tandem Repeats Database. *Nucleic Acids Res*. 2007;35(December 2006):80-87. doi:10.1093/nar/gkl1013
196. Madden T. The BLAST Sequence Analysis Tool. The NCBI Handbook. https://www.ncbi.nlm.nih.gov/books/NBK21097/. Published 2002.
197. Zhao S, Jiang E, Chen S, et al. PiggyBac transposon vectors : the tools of the human gene encoding. *Transl lung cancer Res*. 2016;5(1):120-125. doi:10.3978/j.issn.2218-6751.2016.01.05
198. Ivics Z, Mátés L, Yau TY, et al. Germline transgenesis in rodents by pronuclear microinjection of Sleeping Beauty transposons. *Nat Protoc*. 2014;9(4):773-793. doi:10.1038/nprot.2014.008
199. Knight CH, Peaker M. MAMMARY CELL PROLIFERATION

IN MICE DURING PREGNANCY AND LACTATION IN RELATION TO MILK YIELD. *J Exp Physiol*. 1982;67:165-177.
200. Wellenstein MD, Coffelt SB, Duits DEM, et al. Loss of p53 triggers WNT-dependent systemic inflammation to drive breast cancer metastasis. *Nature*. 2019. doi:10.1038/s41586-019-1450-6
201. Na B, Yu X, Withers T, et al. Therapeutic targeting of BRCA1 and TP53 mutant breast cancer through mutant p53 reactivation. *npj Breast Cancer*. 2019;(August 2018). doi:10.1038/s41523-019-0110-1
202. Derksen PWB, Braumuller TM, Burg E Van Der, et al. Mammary-specific inactivation of E-cadherin and p53 impairs functional gland development and leads to pleomorphic invasive lobular carcinoma in mice. *Dis Model Mech*. 2011;4:347-358. doi:10.1242/dmm.006395
203. Roninson IB, Broude E V, Chang B. If not apoptosis, then what? Treatment-induced senescence and mitotic catastrophe in tumor cells. *Drug Resist Updat*. 2001;4:303-313. doi:10.1054/drup.2001.0213
204. Barker N. Adult intestinal stem cells : critical drivers of epithelial homeostasis and regeneration. *Nat Rev*. 2014;15(1):19-33. doi:10.1038/nrm3721
205. Schvartzman JM, Sotillo R, Benezra R. Mitotic chromosomal instability and cancer : mouse modelling of the human disease. *Nat Rev*. 2010;10(FeBRuARy):102-115. doi:10.1038/nrc2781
206. García-higuera I, Manchado E, Dubus P, et al. Genomic stability and tumour suppression by the APC / C cofactor Cdh1. *Nat Cell Biol*. 2008;10(7). doi:10.1038/ncb1742
207. Taylor AM, Shih J, Ha G, et al. Genomic and Functional Approaches to Understanding Cancer Aneuploidy. *Cancer Cell*. 2018:1-14. doi:10.1016/j.ccell.2018.03.007
208. Zhang B, Hsu Y, Biology R, Cell HS. Emerging roles of transit-amplifying cells in tissue regeneration and cancer. *Wiley Interdiscip Rev Dev Biol*. 2017;6(5):1-20. doi:10.1002/wdev.282.Emerging
209. Jonkers J, Meuwissen R, Gulden H Van Der, Peterse H, Valk M Van Der, Berns A. Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse. *Nat Genet*. 2001;29:418-425. doi:10.1038/ng747
210. Holland AJ, Cleveland DW. Boveri revisited : chromosomal instability , aneuploidy and tumorigenesis. *Nat Rev Mol Cell Bio*.

2009;10(July):478-487. doi:10.1038/nrm2718
211. Würzburg PG zu. Verhandlungen der Physikalisch-Medizinischen Gesellschaft zu Würzburg. *Univ Johann Christ Senckenb.* 1902;35(67):90.
212. Vasquez RJ, Howell B, Wadsworth P, Cassimeris L. Nanornolar Concentrations of Nocodazole Alter Microtubule Dynamic Instability In Vivo and In Vitro. *Mol Biol Cell.* 1997;8(June):973-985.
213. Orr B, Compton DA. A Double-Edged Sword: How Oncogenes and Tumor Suppressor Genes Can Contribute to Chromosomal Instability. *Front Oncol.* 2013;3(June):1-14. doi:10.3389/fonc.2013.00164
214. Stolz A, Ertych N, Bastians H. Microtubule plus tips : A dynamic route to chromosomal instability. *Mol cell Oncol.* 2015;2(2):1-2.
215. Bakhoum SF, Genovese G, Compton DA. Deviant Kinetochore Microtubule Dynamics Underlie Chromosomal Instability. *Curr Biol.* 2009;19(22):1937-1942. doi:10.1016/j.cub.2009.09.055